Development of the embryo sac and endosperm in some seedless persimmons

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(with plate 16)

Some time ago observations were made on the development of seedless persimmons (*Diospyros virginiana* L.) in the neighborhood of Indiana University. Two or three questions then suggested have been at least partly answered as the result of further investigation. In addition certain facts have been obtained in regard to the development of the embryo sac and endosperm, which may be of sufficient interest to merit publication. After a brief report on the subject in the Proceedings of the Indiana Academy of Science, 1908, other work prevented further studies along that line until this winter.

All the persimmon trees found in the vicinity of Indiana University are strictly dioecious. The pistillate flowers have aborted stamens and the staminate flowers have rudimentary carpels. In none of the former is there a suggestion of the development of pollen. Pollination must then be effected through the agency of insects, particularly bees, which frequent the blossoms in great numbers. Some pistillate trees are as far as three or four miles from any known staminate ones, consequently as large a number as 70 per cent or 80 per cent of the flowers frequently fail to be pollinated. These develop fruits usually of a smaller size but otherwise not of inferior quality. The relative number of seedless berries on an individual tree varies from year to year. Neither is there any special localization of the two kinds except that fewer seeds are found on the lower branches. The fruits developed in the near vicinity of staminate trees are usually quite full of seeds, seldom any being entirely without.

As suggested above, a larger fruit is usually developed from those flowers in which pollination has occurred, but there are many trees on which a large per cent habitually fail to be polli-

nated. The fruits of the latter, however, continue to develop and the integuments of the ovule often enlarge considerably into flat thin seedlike bodies. The structures in the ovules are very hard to procure in proper sections for staining, due to the very hard nature of the integuments. Therefore great care was exercised in arriving at conclusions in regard to the presence or absence and the nature of certain structures in the embryo sac.

The sections for microscopical studies were all made from ovaries that would evidently have formed seedless fruits, as no indications of pollen tubes were evident, and preparations were examined which included the earlier stages in the development of the embryo sac as well as later ones showing the parts nearly or quite degenerate. The considerations then in the following paper must be taken to refer to "seedless persimmons," as none of the material on hand contained evidence of embryonal development, and later observations showed that the trees from which the material was taken bore a very high percentage of seedless fruits.

THE DEVELOPMENT OF THE EMBRYO SAC

Only a small nucellus is formed, PLATE 16, FIG. 13 representing its greatest development but showing no indications of a spore mother-cell or spores. The earliest stage observed in the development of the embryo sac is shown in FIG. 1. Only two nuclei are present and these are near the chalazal end. Whether this condition represents a normal stage or an incomplete development I am unable to say, for many preparations of incompletely developed embryo sacs were found. The growth of the embryo sac takes place well toward the micropylar end of the nucellus, all of this portion eventually breaking down, leaving a small but quite persistent remnant at the opposite extremity. FIG. 2 shows a condition frequently met with. The egg apparatus seems quite normally developed and the polar nuclei are closely associated. The two latter have not been observed in an actual state of fusion although some preparations show the contiguous sides to be somewhat flattened (FIG. 4). Other sections of the same ovule (FIG. 2) show one or two antipodal cells, but frequently these are not of definite form or constant in number. FIG. 3 shows three antipodal cells all present in the same section. Only two cells

of the egg apparatus are shown but the third cell is present in the succeeding section of the series. Traces of the polar nuclei are present, one of which lies in the neighboring section but the other one is apparently disorganizing. No later stage in the fusion of the polar nuclei was observed than that shown in FIG. 4, where they are merely very closely associated. FIG. 5, 6, and 7 represent a condition which seems difficult of explanation and yet is consistent with facts brought out in FIG. 8–11. By following through the successive sections of this ovule (FIG. 5–7) three nuclei were found in the region usually occupied by the egg apparatus. FIG. 5 shows one nucleus lying in a large cell not unlike an egg cell. FIG. 6, which is the succeeding section, contains a nucleus in a denser cytoplasmic mass nearer the periphery of the embryo sac, and the next section shows the third nucleus in a still denser mass at nearly the opposite side of the sac. This latter mass appears to be of a mucilaginous nature, only a portion of it differentiating like the ordinary cytoplasm of the egg apparatus. FIG. 6 and 7 do not appear to represent either egg cell or synergids but resemble more nearly disorganizing nucellar tissue. (Compare FIG. 1 and 4.) However, FIG. 5–7 may merely represent a breaking down egg apparatus consequent to the failure of fertilization. Disorganizing nuclei of the nucellar tissue lie in the peripheral cytoplasm of the embryo sac.

I am convinced that a complete embryo sac is not always organized, and that even in the absence of fertilization, whether completely organized or not, development in the sac does not always cease at this point. FIG. 8 and 9, drawn from the micropylar end of the embryo sac, seem to be a slightly more advanced stage. They represent neighboring sections of the same ovule but two distinct groups of cells. The cells of FIG. 8 more nearly resemble those of the egg apparatus than do those of FIG. 9. A similar condition is shown in another ovule (FIG. 10). Neither evidence of fertilization nor development of the egg were discovered at this or later stages, but nuclei appear around the periphery and somewhat more numerous in the micropylar region of the embryo sac. The entire history of these nuclei has not been traced but their appearance would suggest that they may have originated from the polar nuclei. That they represent an early stage of

endosperm formation is quite clear. (Compare FIG. 10, 11, and 12.) Development proceeds more rapidly in the micropylar portion, which may be for a time separated from the opposite or chalazal region (FIG. 11). The cellular endosperm continues its inward development until the entire cavity is filled. In the region formerly occupied by the egg there are usually remnants of broken down tissue (FIG. 12). Although the sections were all examined carefully, there were no indications of a pollen tube having penetrated, nor of an embryo at least as far as the ovule.

The development of the endosperm in the seedless persimmon agrees with that noted within recent years for a few other plants but not conforming to the generally accepted theory of endosperm development in angiosperms.

Juel ('98) found that the polar nuclei in *Antennaria* come close together but do not fuse. On the contrary they separate and form endosperm at the same time that the egg parthenogenetically develops an embryo.

Coulter ('98), in *Ranunculus multifidus*, found free nuclear formation of endosperm before fertilization. He suggests that the presence of the pollen tube in the pistil may cause this premature formation. Practically the same conditions with no evidence of fertilization were observed by Smith ('98) in *Eichhornia crassipes*.

Overton ('02) discovered along with parthenogenesis in *Thalictrum purpurascens* the endosperm nucleus always dividing before the egg. Johnson ('02) found similar conditions in *Piper medium* and *Heckeria umbellata*.

Coker ('07) observed endosperm development in *Pontederia cordata* and *Heteranthera limosa* taking place in two distinct regions, a smaller antipodal and a larger micropylar. I find that somewhat the same condition may obtain in the seedless persimmon, except that the chalazal portion in the latter is relatively much larger than those figured by Coker for *Pontederia* and *Heteranthera*. The figures of Smith ('08) show a striking similarity to my FIG. 10 and 11.

CONCLUSIONS

That any of these features are constant for the seedless persimmon is not at all certain, on the contrary there seems to be

a great variation. A fully developed embryo sac is not always formed before the parts begin to disorganize. There is a tendency toward incomplete organization especially of the antipodal cells. The egg apparatus is, of all the parts observed, most frequently in a state of complete organization but it too may break up prematurely. The polar nuclei are often observed in close contact but never in a state of fusion. The integuments frequently push in at this stage and obliterate the cavity of the embryo sac without further development of the latter. However, considerable endosperm tissue may be produced while at the same time the cells of the egg apparatus disorganize. This development of endosperm is more rapid in the region of the micropyle and may be separated at first from the tissue in the opposite portion. At first free nuclei are observed scattered in the peripheral layer of cytoplasm, then cell formation begins and gradually extends toward the center until the cavity of the embryo sac is completely filled with a cellular endosperm tissue.

It may be of interest also to note that a very defini e nutritive jacket layer of cells is organized from the inner integument (FIG. 3 and 11), and that, although only a small nucellus is formed (FIG. 13), a relatively large portion of it is quite persistent.

It remains an open question as to the stimulus causing the occasional development of endosperm without fertilization. There is the possibility of the presence of pollen tubes in the upper part of the pistil, or at least outside of the ovule, but developing too late to effect fertilization. Whatever may be the stimulus, a well organized endosperm may develop without the initiation of an embryo.

I hope to procure material this summer from trees developing fertile seeds in order to trace the embryology. Experimental plantings show that a large per cent of the well developed seeds germinate readily.

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Explanation of plate 16

FIG. 1. A two-nucleated embryo sac, upper part of nucellus disorganizing. × 450.

FIG. 2. Egg apparatus and polar nuclei. One or two antipodal cells were present in the next section. × 450.

FIG. 3. Embryo sac showing antipodals. Upper part of nucellus has disappeared. × 450.

FIG. 4. Egg apparatus. Polar nuclei flattened on contiguous sides. × 525.

FIG. 5, 6, and 7 are successive sections of the same ovule. × 150.

FIG. 6 and 7 represent only the micropylar region.

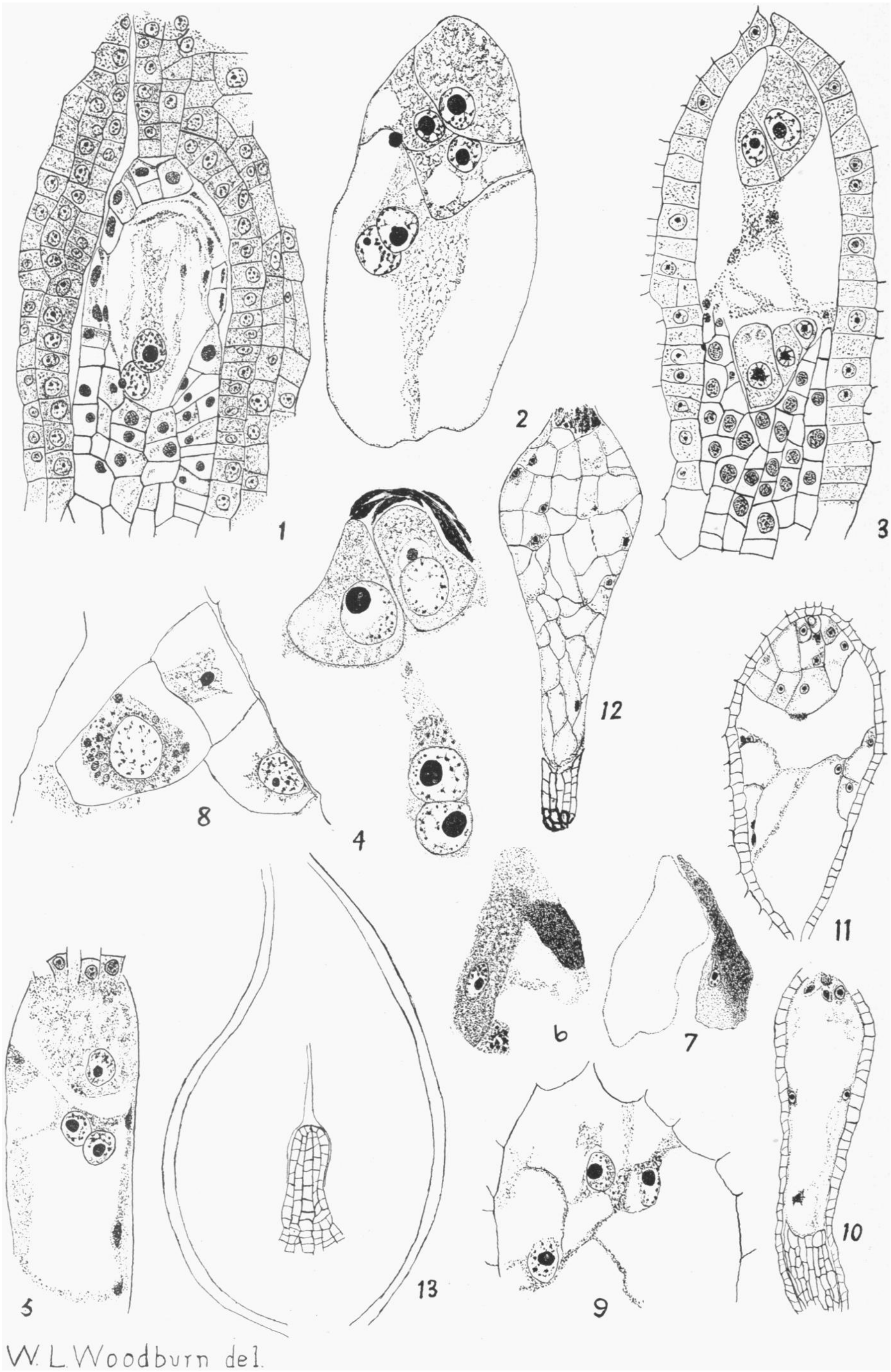
FIGS. 8 and 9 are successive sections through corresponding regions of an older embryo sac, showing two distinct groups of cells. × 150.

FIG. 10. Section of embryo sac showing peripheral endosperm nuclei and a group of disorganizing cells, evidently the egg apparatus. × 55.

FIG. 11. Showing the development of the endosperm in two regions. × 55.

FIG. 12. Endosperm completely filling the embryo sac. × 55.

FIG. 13. Outline sketch showing extent of nucellus surrounded by inner integument, before formation of megaspore.



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